

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

PCT

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

Date of mailing

(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION

See paragraph 2 below

International application No.
PCT/EP2004/003864

International filing date (day/month/year)
13.04.2004

Priority date (day/month/year)
14.04.2003

International Patent Classification (IPC) or both national classification and IPC
G01R33/46

Applicant
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1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2004/003864

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.
☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
 - a. type of material:
☐ a sequence listing
☐ table(s) related to the sequence listing
 - b. format of material:
☐ in written format
☐ in computer readable form
 - c. time of filing/furnishing:
☐ contained in the international application as filed.
☐ filed together with the international application in computer readable form.
☐ furnished subsequently to this Authority for the purposes of search.
3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY**

International application No.
PCT/EP2004/003864

Box No. II Priority

1. ☒ The following document has not been furnished:

- ☒ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).
- ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	2,4,6,10-15
	No: Claims	1,3,5,7-9
Inventive step (IS)	Yes: Claims	14,15
	No: Claims	1-13
Industrial applicability (IA)	Yes: Claims	1-15
	No: Claims	

2. Citations and explanations

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING
AUTHORITY (SEPARATE SHEET)**

PCT/EP04/03864

- 1 The following documents are referred to in this written opinion, the numbering will be adhered to in the rest of the procedure:**

D1: US 2002/0006382

D2: Bajaj VS et al, J. Magn. Reson. Vol. 160, pp 85-90 (2003)

D3: Wind RA et al, Advances in chemistry series, American chemical society, Vol. 229, pp 45-63 (1993)

D4: Lyon CE et al, J. Am. Chem. Soc. Vol. 121, pp 6505-6506 (1999)

D5: Zhao L et al, Proc. Int. Soc. Magn. Reson. Med. p. 451 (1998)

D6: Zhao L et al, J. Magn. Reson. B, Vol. 113, pp 179-183 (1996)

2 Re Item V: Reasoned statement under Rule 43bis.1(a)(i) PCT

With respect to the objection under Art. 6 PCT given below (see item 3.1 below), lines 6-11 of claim 1 were replaced by the following clarified features to compare the defined subject-matter with the prior art:

- performing NMR spectroscopy on the sample with the use of sequences of rf pulses, wherein the pulse sequences **comprise** at least two rf pulses, either on the same nuclei or on different nuclei, and wherein **both** pulse sequences is adapted for a hyperpolarized sample, thereby producing at least two **one-dimensional NMR spectra or at least one multidimensional NMR spectrum**.
- analysing at least two of the NMR spectra **or the at least one multidimensional NMR spectrum** in order to obtain a characterisation of the sample, or to obtain an interim result to be used in the NMR spectroscopy step.

The search was also based on this clarification.

Accordingly, claim 3 is clarified in that "the pulse sequences **are** adapted ... by using repeated excitation pulses with small flip angles ...".

2.1 Lack of novelty and/or an inventive step (Art. 33(2) and 33(3) PCT)

Claim 1

The subject-matter of claim 1 would appear to lack novelty with respect to each of the documents D1-D4 for the following reasons.

For instance, document D1 discloses (references in parentheses referring to D1):

A method of performing nuclear magnetic resonance spectroscopy on a hyperpolarized sample (see par. [0002], which method comprises the steps of:

- hyperpolarizing a sample using DNP, wherein the NMR active nuclei receive hyperpolarization (D1 implicitly discloses an embodiment wherein hyperpolarization of ^{129}Xe is performed using DNP, see par. [0080]; moreover, ^{129}Xe represents the "NMR active nuclei", see for instance figures 10A-10P and par. [0178]);
- performing NMR spectroscopy on the sample with the use of sequences of rf pulses (see par. [0178] and figures 10A-10P), wherein the pulse sequences comprise at least two rf pulses, either on the same nuclei or on different nuclei (each of the pulse sequences comprises one 20 degree excitation pulse applied to ^{129}Xe , see par. [0178]; furthermore, since a plurality of pulse sequences is applied consecutively (see the time series depicted in figures 10A-10P), it is considered that the pulse sequences comprise at least 2 rf pulses), and wherein the pulse sequence is adapted for a hyperpolarized sample (the use of excitation pulses with a flip angle of 20 degrees (see par. [0178]) is considered to be "adapted for a hyperpolarized sample", see also par. [0174]), thereby producing several two one-dimensional NMR spectra (the plurality of NMR spectra depicted in figures 10A-10P);
- analysing at least two of the NMR spectra in order to obtain a characterization of the sample, or to obtain an interim result to be used in the NMR spectroscopy step (from the plurality of NMR spectra depicted in figures 10A-10P the sample is characterized in that it is concluded that the hyperpolarized "gas remained substantially in the gas phase", see par.

[0178]).

In a similar way, the lack of novelty can be shown with respect to documents D2-D4 (see the passages of these documents cited in the search report).

2.2 Claims 2-13

Claim 2

The subject-matter of claim 2 differs from that of claim 1 only in that an initial 90° pulse followed by a plurality of spin echo pulses is applied rather than a small flip angle pulse as in D1. The corresponding technical effect is an increased signal-to-noise ratio (SNR) of the measurement since the initial 90° pulse generates a higher transverse magnetization than a small flip angle pulse. However, it would appear that the skilled person, desiring to increase the SNR of the measurement, would consider document D5 since the same problem is mentioned therein (see D5, section "Introduction", lines 13-15). The problem is solved in D5 by using a single scan pulse sequence in which one initial 90° pulse is followed by a plurality of spin echo pulses (see section "Introduction", lines 20-29 and section "Methods"). Therefore, it appears that the skilled person would arrive at the subject-matter of claim 2 without the exercise of any inventive skill.

Claims 3, 5

The additional features of claims 3 and 5 are known from D1 as well (see par. [0178]).

Claims 4, 6

The subject-matter of claim 4 differs from D1 in that the flip angle of the excitation pulse is varied rather than kept constant as in D1. However, the advantages of varying the flip angle in the context of an MR experiment involving a hyperpolarized sample are well-known in the art (see e.g. D6). Therefore, it is considered that the skilled person, desiring to provide a constant transverse magnetization throughout the MR experiment, would vary the flip angle of the excitation pulse according to document D6 and arrive at the subject-matter of claim 4 without the exercise of any inventive skill.

Furthermore, the additional features of claim 6 are known from document D6 as well (see section "Simulation", 1st paragraph; it is noted that the statement that the "flip angle sequence ... uses all the available magnetization" implies that the last excitation pulse is a 90° pulse). Therefore, the subject-matter of claim 6 lacks an inventive step with respect to a combination of documents D1 and D6 as well.

Claims 7-9

The additional features of claims 7-9 are disclosed in each of the documents D2 and D4 (see for instance D2, figures 4 and 5; w.r.t. claim 8 it is noted that the experiment disclosed in D2 can be considered to explore the multidimensional time space "through a trajectory that spans all parts ... that provide essential information"; w.r.t. claim 9 see the two-dimensional experiment disclosed in figure 4 of D2).

Claim 10

Claim 10 would appear to define a simplified version of a heteronuclear COSY experiment wherein two one-dimensional spectra rather than one two-dimensional spectrum are acquired. However, a two-dimensional **homonuclear** COSY experiment is disclosed in document D2 (see e.g. figure 4). Furthermore, the possibility of performing **heteronuclear** COSY experiments to correlate the chemical shifts of different kinds of nuclei in order to determine which nuclei are directly bonded to one another is well-known in the art and even D2 itself hints at the possibility of performing "multidimensional correlation experiments" (D2, page 89, last paragraph).

Moreover, the simplification of acquiring two one-dimensional spectra rather than one two-dimensional spectrum cannot be considered to involve any inventive skill since it is well-known in the art that all information that is present in two one-dimensional spectra (and even more) is also accessible via a corresponding two-dimensional spectrum. Therefore, it is considered that the subject-matter of claim 10 lacks an inventive step with respect to document D2.

Claims 11, 12

Claims 11 and 12 specify that the spectroscopy step comprises decoupling of the first and second nuclei. However, spin decoupling in order to simplify NMR spectra by removing the splittings caused by scalar coupling among the decoupled nuclei is well-known in the art (documents will be cited if necessary). Therefore, it would appear that the subject-matter of claims 11 and 12 does not involve any inventive step with respect to document D2 as well.

Claim 13

Claim 13 specifies that the pulse sequence of the NMR spectroscopy method is diffusion weighted. However, this possibility, its implementation using bipolar magnetic field gradients and its advantages are well-known in the art (documents will be cited if necessary). Therefore, it is considered that the skilled person, for instance starting from D2 and desiring to characterize the interaction between proteins and ligands, would add diffusion weighting to the NMR experiment disclosed in D2 and arrive at the subject-matter of claim 13 without the exercise of any inventive skill.

Novelty and inventive step of claims 14, 15

Claims 14 and 15 specify particular trajectories through a multidimensional time-space in the context of a multidimensional NMR spectroscopy method on a hyperpolarized sample. None of the prior art documents cited in the search report addresses the problem of selecting a particular trajectory at all. Therefore, it would appear that the skilled person finds no indication in the prior art to perform a multidimensional NMR spectroscopy experiment that involves the particular trajectories defined in claims 14 and 15, respectively. Thus, the subject-matter of claims 14 and 15 is considered to be novel and inventive.

3 Re Item VIII: Lack of clarity and support in the description

3.1 Claim 1

- a) The wording "pulse sequences comprises at least two rf pulses" is obscure. However, with respect to the embodiment disclosed on pages 9-11 of the description wherein two one-dimensional spectra of ^1H and ^{13}C are recorded, respectively, it would appear that **each** pulse sequence comprises **only one** rf pulse.
- b) The wording "thereby producing at least two NMR spectra" would appear to represent a contradiction to the embodiments given on pages 14-18 of the description wherein only **one** two-dimensional spectrum is produced.
- c) It is not apparent what is to be understood by the term "an **interim result** to be used in the NMR spectroscopy step".
- d) Moreover, the wording recited in item d) is obscure since the "spectroscopy step" has already been finished when the "interim result" is obtained. Therefore, it is unclear how the "interim result" can "be used in the NMR spectroscopy step".

3.2 Claim 2

It is unclear whether "spin echo pulses" correspond to inversion pulses that flip the magnetization by an angle of 180° .

3.3 Claim 3

The scope of the relative term "**small flip angles**" is unclear. The same objection applies to claim 12 as well.

3.4 Claim 4

The broad scope of the wording "flip angle ... is varied" appears not to be supported by the description. The description merely supports that the flip angle is varied such that an equal amount of transverse magnetization is produced throughout the series of "repeated excitation pulses with small flip angles" (see the description, page 13, lines 19-24). Therefore, the features of claim 6 should have

been added to claim 4.

3.5 Claim 5

The scope of the wording "**essentially** constant" is unclear. Therefore, the word "**essentially**" should have been deleted.

3.6 Claim 7

- a) There appears to be a contradiction between claim 7 (defining a multidimensional NMR spectroscopy experiment) and claim 1 (defining that "at least two NMR spectra" are produced) since a multidimensional NMR spectroscopy experiment normally provides only one multidimensional NMR spectrum.
- b) It is unclear whether the term "pulses" in line 28 refers to rf pulses, gradient pulses, etc.
- c) The term "the time parameters" lacks an antecedent definition.
- d) It is unclear whether the feature defining that "said pulse sequence being adapted to take into account the initial hyperpolarized spin state" corresponds to the specification in claim 1-3 defining that "the pulse sequence is adapted for a hyperpolarized sample".

3.7 Claim 8

It is not apparent what is to be understood by the term "essential information". Therefore, claim 8 is obscure.

3.8 Claim 9

It is not apparent what is to be understood by the term "two time plane". It would appear that this term corresponds to the "multidimensional time space" defined in claim 7.

3.9 Claim 10

The wording "identifying in the first spectra" is obscure since only one "first ... spectrum" is recorded.

3.10 Claims 14, 15

It is not apparent what is to be understood by the point coordinates (e.g. "point (0,0)", "points (i,i)", "points (N,N)") since the underlying space has not been defined.

3.11 Description and drawings

- a) The statements throughout the description according to which certain documents are "incorporated by reference" should have been deleted.
- b) The labeling of figures 2a and 2b appears to be incorrect (see page 12 of the description).
- c) The speculative broadening by referring to a "future method of hyperpolarization" on page 6, line 24 should have been deleted.